European Journal of Nutrition & Food Safety

8(1): 29-46, 2018; Article no.EJNFS.2018.005
ISSN: 2347-5641

Effect of Arabic Gum as Prebiotics and *Lactobacillus casei Shirota* (LcS) as Probiotic on Oxidative Stress and Renal Function in Adenine–Induced Chronic Renal Failure in Rats

Fadl Alla, Eman Aly Sadeek[1*]

[1]Department of Biochemistry and Nutrition, Women's College, Ain Shams University, Cairo, Egypt.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/EJNFS/2018/36022

Original Research Article

Received 8th August 2017
Accepted 12th January 2018
Published 24th January 2018

ABSTRACT

Objectives: This study aimed at assessing the effect of Gum Arabic as Prebiotics and lactobacillus casei shirota (LcS) as probiotic on oxidative stress and renal function in adenine–induced chronic renal failure in rats.

Methodology: 70 male albino rats were divided into 7 groups and treated for 8 weeks as follows group 1: control basal diet group (BD), group 2: adenine in feed (0.75%, w/w), group 3: gum Arabic (GA) in drinking water (15%, w/v), group 4: lactobacillus casei shirota (LcS) 1 x 10^9 colony-forming units (CFU) supplement, group 5 adenine + GA, as before, group 6 adenine +(LcS) as before and group 7: adenine + GA+ (LcS) as before. Urine, blood and kidneys were collected from the rats at the end of the treatment for analysis of conventional renal function tests serum creatinine, urea, uric acid, sodium, potassium concentration). In addition, the oxidative stress markers serum and kidney glutathione and superoxide dismutase, serum catalase and malondialdhyde (MDA) were measured.

Results: By the end of the 8 weeks of treatment, Adenine significantly ($p < 0.05$) increased the concentrations of serum creatinine, urea, uric acid, sodium, potassium and serum MDA. In addition, the oxidative stress markers serum and kidney glutathione and superoxide dismutase, serum catalase was, significantly decreased.

Treatment with (GA) and (LcS) significantly ameliorated these actions. The mechanism of the reported salutary effect of GA in adenine-induced CRF is associated with mitigation of the adenine-induced inflammation and generation of free radicals.

Conclusion: The results suggest that that oral administration of gum Arabic and lactobacillus casei shirota could conceivably alleviate adverse effects of adenine induced renal toxicity (CRF).

**Corresponding author: Email: dremanfadlalla@gmail.com;*

Keywords: Gum Arabic; prebiotics; Lactobacillus casei Shirota (LcS); probiotic; oxidative stress; adenine; chronic renal failure.

1. INTRODUCTION

Chronic kidney disease (CKD) is a serious worldwide health problem and is now considered a main determining factor of the poor health consequences of major non-infectious diseases [1]. Numerous elements influence the onset and development of this CKD, such as obesity, diabetes mellitus, and hypertension. Further, then these factors, there is evidence of a pathophysiological role for inflammation and oxidative stress in CKD and its complications [2]. These two events are noticeable features of CKD and its complications in human being [3]. Elevated oxygen radical formation was Found in CKD [4]. Therefore, there is a strong need for new research discovering new therapeutic strategies and ameliorating agents, especially in the earlier stages of CKD to slow its progression towards ESRD. Among these agents are Gum Acacia (GA) and *Lactobacillus casei Shirota* (LcS).

Adenine is used in this study as one of the models used to induce chronic kidney diseases (CKD) in rats. Both adenine and its metabolite, 2,8-dihydroxyadenine (DHA), have low solubility and can precipitate in the renal tubules and form crystals [5].

Arabic Gum (GA) is extracted from exudates of Acacia seyal trees. It comprises of a blend of polysaccharides in addition to oligosaccharides and glycoproteins [6]. Sudan is the world's biggest producer, trailed by numerous other African nations. It promptly disintegrates in water forming low viscosity solutions [7].

Gum Arabic is indigestible to human and animals, but rather fermented in the colon to provide short-chain unsaturated fats, stimulating numerous medical advantages. One of these advantages is its prebiotic impact. It has been demonstrated that four-week supplementation with Gum Arabic (10 g/day) prompted critical increments in Bifidobacteria, Lactobacteria, and Bacteroides demonstrating a prebiotic impact [8].

The intestinal microflora of humans and animals is a complex ecosystem that contains several species of useful and hurtful microorganisms. Probiotics' are well-defined as living microorganisms, which confer a benefit on the host, when given in adequate quantities, [9].

Probiotics have potential dietary supplement uses (to help maintain healthy organ functions), and drug uses (to prevent or treat disease).

Lactobacilli are gram-positive bacteria characterized by the formation of lactic acid. Numerous Lactobacillus species are also members of the normal microbiota of animal and human gastrointestinal and genitourinary tracts and are reported to have many health benefits. [10].

This study was designed to examine the consequences of oral administration of Gum Arabic as Prebiotics and Lactobacillus casei Shirota (LcS) as probiotic in rats. Specifically, this involved:(a) assessing kidney function, (b) assessing Oxidative Stress in adenine-induced chronic renal failure rat model.

2. MATERIALS AND METHODS

2.1 Animals

Healthy Male Wistar rats (weighing 190.20g ± 5.91) were obtained from the Institute of Ophthalmology (Cairo, Egypt). They were allowed to acclimatize for two weeks. The animals were housed in stainless steel cages at a temperature of (25 ± 3°C), and light- controlled room (12-12 h), relative humidity of about 60%, w, during acclimatization period animals fed on basal standard diet prepared in accordance with AIN-93 formulation [11] (Reeves et al. 1993) and provided with water *ad libitum*.

2.2 Chemical and Drugs

Adenine was obtained from Sigma (St. Louis, MO, USA). Adenine was prepared freshly in 10% Tween 80 obtained from El Gomhouria Co., Zagazig, Egypt. Acacia gum used obtained from Gum Arabic Company in Khartoum, Sudan and verified Aqueous solutions of Acacia gum used were prepared freshly every day. All used chemicals were of analytical reagent grade. Lactobacillus casei Shirota was provided by Yakult United Arab Emirates (U.A.E).

2.3 Estimation of the Total Phenolic and Total Flavonoids Contents of GA

Gum Arabic (GA) was dissolved in distilled water to prepare (15%, w/v) solution. Afterwards, the (15%, w/v) solution was used for analysis. Total phenolic content (TP): TP contents were estimated using the Folin–Ciocalteu reagent as described by Al-Farsi et al. [12]. The calculation was based on a calibration curve obtained with gallic acid. The TPC was expressed as mg gallic acid equivalent (GAE) / g of dry material (DM).Total flavonoid (TF): TF contents were determined according to the method of Zhishen et al. [13]. The standard curve (0.05–0.5 mg gallic acid / mL), and the results were expressed as mg gallic acid equivalent (GAE) per g DM.

2.4 The Experimental Design

After an acclimatization period of 2 weeks to stabilize all metabolic conditions, rats (n= 70) were randomly divided into seven equal groups and treated for eight consecutive weeks as shown in the following Table 1.

2.5 Handling of Blood, Urine and Kidney Samples

During the treatment, period the rats were weighed weekly, For the collection of urine, they were placed individually in metabolic cages for 24 h, after the 8 weeks' treatment period. Urine specimens were centrifuged at 900 rpm for 15 min, (The supernatant remains after centrifugation was transferred by careful decanting or pipetting. It is important that the sediment is not disturbed whilst the supernatant is being transferred) and then urine volumes were measured. Urine specimens were stored at -20°C until analyzed for creatinine, total proteins, as well as sodium, potassium and calcium electrolytes. At the end of the 8 weeks' experimental period, the rats were fasted overnight and sacrificed under ether anesthesia, and blood samples were collected from the hepatic portal vein. The blood samples were centrifuged at 1500 rpm for 15 min. The serum samples were stored at -20°C for further assessment. The kidneys were excised, rinsed with saline (0.9% NaCl) blotted on filter paper and weighed and stored -20°C for subsequent analysis.

2.6 Biological Assay

The rats were weighed weekly, and food intake was estimated The Feed Efficiency Ratio (FER) was calculated as described by the equation of Guo et al. [17].

Feed efficiency ratio (FER) = weight gain (g) / feed intake (g).

2.7 Biochemical Assays

The concentrations of creatinine in serum and urine were estimated spectrophotometrically using commercial kits [18] Creatinine clearance (CCr) was calculated as reported by Duarte et al. [19]. Urea was estimated according to Fawecett and Scott [20], while uric acid was

Table 1. The experimental design

Group	Treatment
Group 1 (Control group) "C"	Continued to receive the same standard diet without treatment until the end of the study
Group 2 feed (AD)	Received standard diet containing adenine at a dose of 0.75%w/w in feed (AD)
	Was given standard diet and GA in drinking water at a concentration of 15% w/v (GA)
Group 4 (LcS)	Received lactobacillus casei Shirota (LcS) supplement orally 1 x 10^9 colony-forming units (CFU)/day in the formulation (LcS)
Group 5 (AD+GA)	The fifth group was given adenine in the feed as in group 2, plus GA in drinking water at a concentration of 15% w/v (AD+GA)
Group 6 (AD+LcS).	Received standard diet containing adenine at a dose of 0.75%w/w in the feed (AD) + (LcS) as before (AD+LcS).
Group 7 (AD+GA+ LcS)	Adenine + GA+ (LcS) as before. (AD+GA+ LcS)

The dose of adenine was chosen from the original method by Yokozawa et al. [14] and the dose of GA according to [15], the lactobacillus casei Shirota (LcS) dose was according to Karimi et al. [16]

determined by enzymatic colorimetric kits Gochman and Schmitz [21]. Proteinuria was measured with a kit according to Tietz [22]. serum and urinary potassium concentrations were determined by [23], while serum and urinary sodium concentrations were measured colorimetrically using kits according to] [24], Calcium was determined in the Serum according to Baginski, [25].

All the previous parameters were estimated using commercial kit (Cayman Chem. Co., Michigan, USA) serum and renal malondialdehyde (MDA) were estimated according to Ohkawa et al. [26], while Superoxide dismutase (SOD) were determined according to Minami and Yoshikana [27], while reduced glutathione(GSH) were measured chemically according to Beutler et al. [28]. Finally, catalase (CAT) activity was determined by colorimetric kits according to [29]. Serum and renal MDA, GSH, and CAT, as well as SOD, were estimated using a commercial kit from Randox Ltd., Co. (UK).

2.8 Statistical Analysis

Data are expressed as mean ± standard Error. Statistical comparison between different groups was conducted using one-way analysis of variance (ANOVA). Statistics were performed using the Statistical Package for the Social Science (SPSS)software version 17.0 was used for all statistical analyzes (SPSS Inc., Chicago, IL, USA). Significance was accepted at P, <0.05. Values were expressed as the Mean ± SEM [30].

3. RESULTS AND DISCUSSION

In vivo animal, biological experiments are of great importance in examining the physiological and biochemical effects of some possible therapeutic intervention on chronic kidney diseases prior to studies on humans. The present research reveals the novel effect of GA as prebiotic and (LcS) as probiotic on kidney function and antioxidant status in adenine-induced renal failure rat models.

Chronic kidney disease (CKD) progresses at variable rates, depending on the etiology. It has been reported that CKD is fatal disorder until the development of renal replacement therapies involving of hemodialysis, peritoneal dialysis, and kidney transplantation allowed decades or longer of survival.

Kidneys dysfunction is related to disturbed kidney metabolism and to reduced glomerular filtration and tubular secretion/reabsorption problems [31]. Recent studies suggested that the bacterial load and the adverse products of the intestinal microbiota might influence chronic renal disease pathogenesis [30].

The development of chronic kidney disease upon adenine oral administration is confirmed by subsequent significant increases in fluid intake and urinary output, in addition to the significant elevation of serum creatinine and urea and the decrease in creatinine clearance as well as in body weight.

The results of the current study showed that GA contained significantly higher levels of phenolic and flavonoid compounds where The total phenolic and total flavonoids contents of GA are as follows: TPC (36.14 ± 0.18 (mg of GAE/g DM)), TFC (21.76 ± 0.04 (mg GAE/g DM) as shown in Table 2 which indicated that GA is a good source phenolic and flavonoid compound, which can be a powerful antioxidant source. The high antioxidant capacity of GA attributed to the active components dissolved in the water. In general, phenolic compounds may be related directly to antioxidant action, and to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. The high phenolic and flavonoids compounds of GA could protect the kidney from adenine toxicity.

Table 2. The total phenolic and total flavonoids contents of GA

Parameter	(mg of GAE/g DM)
Total phenolic content	36.14 ± 0.18
Total flavonoids content	21.76 ± 0.04

Our results are in accordance with those of Negri et al. [32].

The data represented in the Table 3 indicating that adenine significantly reduced the final body weight of rats, and the concomitant administration of GA or /and LcS with adenine has no significant effect on the reduction in body weight.

The relative weight of kidney significantly increased upon adenine administration, and the supplementation with of GA or /and LcS with adenine significantly diminished that increase.

A significant reduction in food intake has been observed by GA or/ and LcS administration which could attribute to the high dietary fiber content of GA which promotes satiety and satiation.

The results of the current study are in harmony with those of Al Za'abi et al. [33] indicating that GA did not significantly modify the body weight of the treated groups. Despite food intake (FI) was significantly decreased after GA treatment.

GA significantly reduces fluid intake and as a result urinary output.

Recent researches highlighted that some bacterial strains, such as *Lactobacillus s*pp. and *Bifidobacterium* spp., play an important role in energy metabolism and weight management in rat models and human being. The beneficial effects of bacteria are strain-dependent [34].

LcS treatment was followed by a significant body weight reduction (Table 2) which reflects energy expenditure prompt [35]; therefore, It has also been suggested that Probiotics can increase the function of the intestinal barrier, leading to body weight loss [36]. In addition, the oral administration of probiotics increases the activity of the sympathetic nervous system in white and brown adipose tissue. Thus, probiotic consumption facilitates thermogenic and lipolytic responses via stimulating the sympathetic nervous system, which leads to weight reduction [37].

The results in a Table 3 revealed no significant effect of feeding GA or LcS in groups 3 and 4 as compared to normal control group 1. In addition the mean feed efficiency ratio (FER) showed non-significant difference in groups 5, 6 and 7 fed GA, LcS, and their combination respectively as compared to adenine – intoxicated group 2 which.which may be attributed to the non significant effect of GA or/and LcS on final body weight in the current study.

By the end of the 8 weeks' experimental period, Adenine tended to significantly ($p < 0.05$) increased the concentrations of serum creatinine (Fig. 1), urea (Fig. 2) and uric acid (Fig. 3). These findings are in accordance with those of Al Za'abi et al. [31] [33] who reported that adenine and its metabolite, 2,8-dihydroxyadenine (DHA), have low solubility and can precipitate the renal tubules and form crystals. Adenine oral consumption might cause the occlusion of renal tubules which hinders the excretion of nitrogenous substances leading to a biochemical and physiological status like chronic kidney disease humans [38].

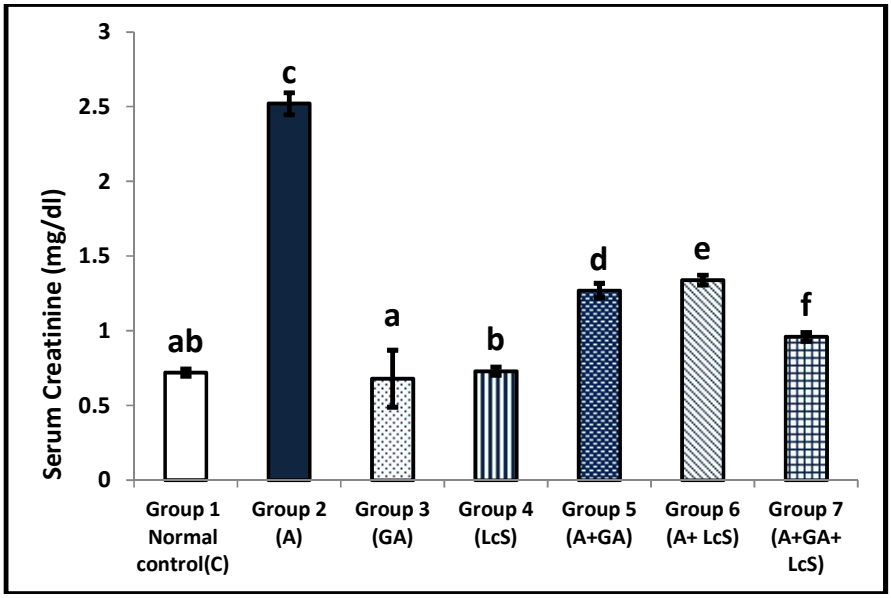

Fig. 1. Serum Creatinine concentration in control and treated rats

Each column and vertical bar represents the mean ± SEM (n = 10). P < 0.05 vs. adenine treatment
Values are expressed as means ± SEM *(n = 10). Means with similar superscript (a, b, c, d) letters indicate the non-significant difference (P< 0.05)*

Table 3. Biological parameters at the end of the experiment

Parameters / Groups	Food intake (g)	Initial body weight (g)	Final body weight (g)	Mean feed efficiency	Relative kidneys weight (%)	Fluid intake (ml/d)	Urine output (ml/d)
Group 1 Normal control (C)	17.30±0.28^{a}	187.23± 6.68^{a}	242.56±7.11^{a}	0.057±0.004^{a}	0.26±0.02^{a}	18.74 ± 0.61^{a}	10.13±0.68^{a}
Group 2 (A)	9.49 ± 0.28^{c}	189.15 ± 5.43^{a}	188.34±4.7^{c}	- 0.007±0.008^{b}	0.52 ±0.03^{c}	25.99 ± 2.06^{d}	21.15±1.67^{c}
Group 3 (GA)	15.96±0.48^{d}	191.89 ± 4.82^{a}	240.07±7.35^{ab}	0.054±0.011^{a}	0.26 ± 0.03^{a}	17.34 ± 0.67^{b}	10.20±0.61^{a}
Group 4 (LcS**)**	17.06±0.29^{a}	190.29 ± 5.47^{a}	236.73±1.85^{b}	0.049±0.008^{a}	0.28 ±0.02^{a}	18.35±0.76^{ab}	10.64±0.66^{a}
Group 5 (A+GA)	8.27± 0.10^{b}	191.36 ± 6.17^{a}	187.84±6.22^{c}	- 0.008±0.015^{b}	0.42 ± 0.07^{d}	23.45 ± 1.67^{c}	14.65±0.54^{b}
Group 6 (A+ LcS**)**	9.19 ± 0.18^{e}	192.31± 5.35^{a}	185.90 ± 5.78^{c}	- 0.012±0.06^{b}	0.39 ± 0.08^{b}	22.51 ± 1.87^{c}	16.08 ±0.65^{d}
Group 7 (A+GA+ LcS)	8.20± 0.18^{b}	189.18 ± 7.23^{a}	186.93 ± 6.52^{c}	- 0.044±0.09^{b}	0.38 ± 0.02^{b}	20.53 ± 1.21^{e}	14.18±0.75^{b}

Values are expressed as means ± SEM *(n = 10). Means with similar superscript (a, b c, d) letters in columns indicate non- significant difference (P< 0.05)*

GA or/ and LcS treatment tended to significantly decrease serum urea, creatinine, uric acid and urinary protein concentrations within the 8 weeks' treatment period as shown in (Figs. 1, 2, 3 and 4) respectively.

On the other hand supplementation with GA or/ and LcS significantly increased Creatinine clearance as compared to adenine intoxicated group.

The results of the present study indicating that adenine supplementation significantly increased serum and urinary sodium and potassium while it caused a significant decrease in serum Ca2+ as illustrated in Table 4. Increased serum calcium concentration upon administration of GA which might be due to increased calcium absorption and retention. This explanation is in accordance with that of who illustrated that **GA** improves calcium absorption and retention in the small intestine [39].

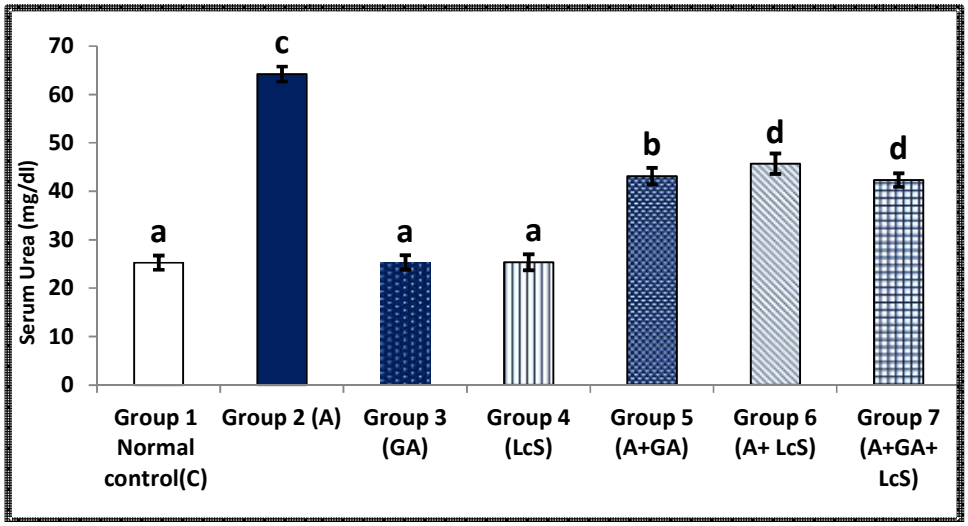

Fig. 2. Serum Urea concentration in control and treated rats

Each column and vertical bar represents the mean ± SEM (n = 10). P < 0.05 vs. adenine treatment

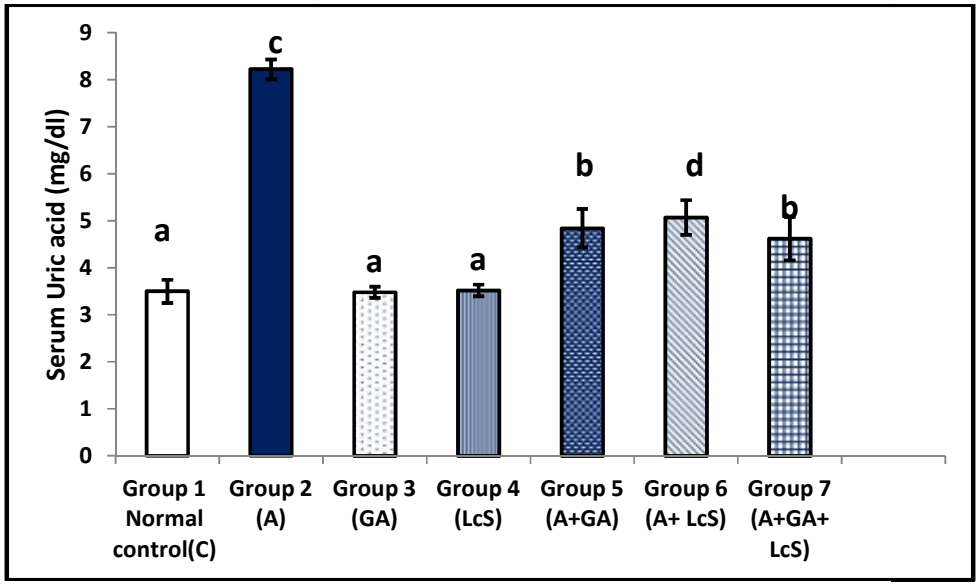

Fig. 3. Serum Uric Acid concentration in control and treated rats

Each column and vertical bar represents the mean ± SEM (n = 10). P < 0.05 vs. adenine treatment

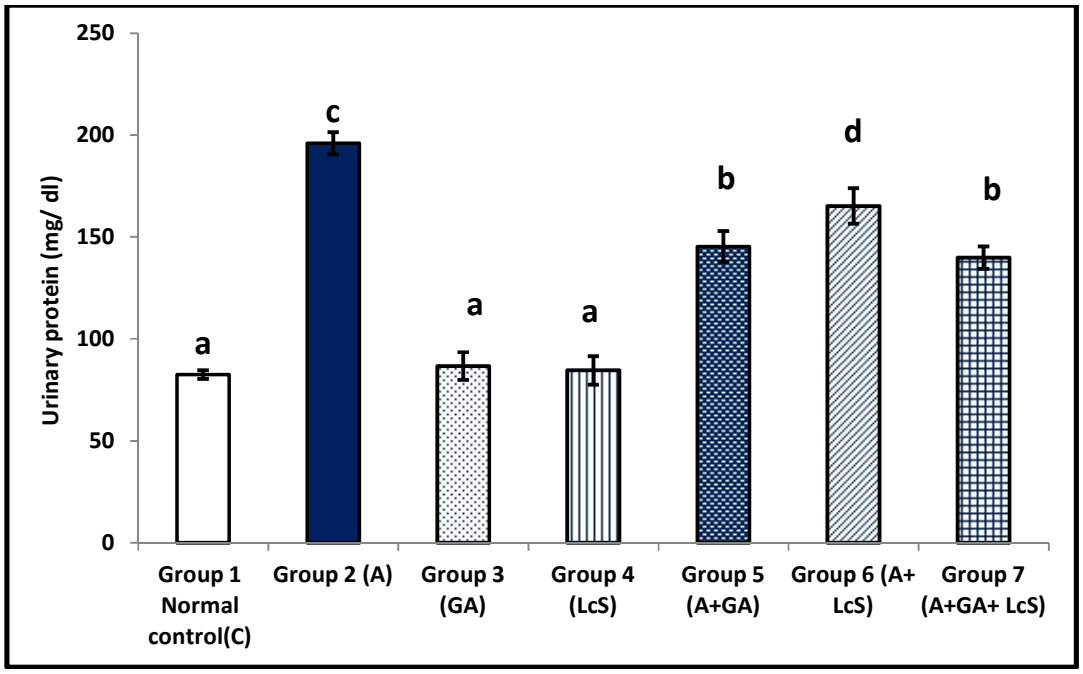

Fig. 4. Urinary Protein concentration in control and treated rats

Each column and vertical bar represents the mean ± SEM (n = 10). P < 0.05 vs. adenine treatment

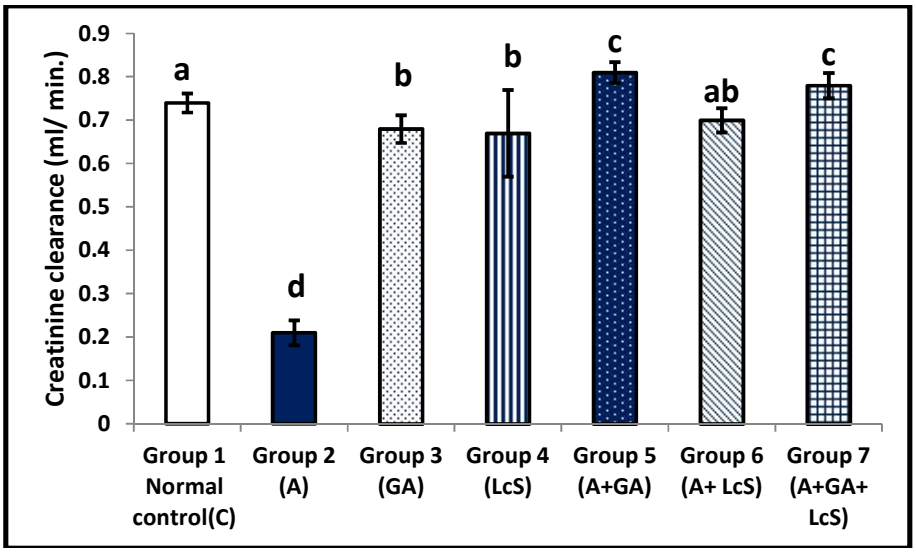

Fig. 5. Creatinine clearance (ml/ min.) in control and treated rats

Each column and vertical bar represents the mean ± SEM (n = 10). P < 0.05 vs. adenine treatment

Urinary Na+ was significantly reduced after GA treatment. On the other hand, the decrease in urinary K+ was not statistically significant.

Serum Na+ and K+ were tended to decrease after GA treatment. On the other hand, GA treatment significantly followed by a profound and significant increase in serum Ca2+.

Gum Arabic (GA), is known to be particularly rich in antioxidants, which have been reported to have antioxidant and lipid peroxidation lowering effects [40].

The nephroprotective effect of GA against many nephrotoxic agents was noted in numerous previous researches [41,42]. The antioxidation induced by GA might be one of the most likely mechanisms contributing to its beneficial effect against renal injury. This antioxidant effect of GA was confirmed previously by in vitro studies, which showed that GA had dose-dependent scavenging of superoxide radicals generated enzymatically and nonenzymatically. It could be suggested that GA scavenges Adenine free-radical generation and, in turn, inhibits lipid peroxidation–induced injury in renal tissues, which has been suggested to protect renal structure and function. Therefore, the protective effect is provided by GA on renal tissue through antioxidants as well as by scavenging free radicals *in vivo*.

GA has many health benefits, including beneficial effects on the kidney. GA is thought to act primarily via increased nitrogen excretion which in turn lowers serum urea nitrogen concentration.

GA is a dietary fiber rich in Ca2+, Mg2+, and K+. It has been demonstrated that GA increased creatinine clearance in healthy mice. Also, it increases ADH excretion, as well as intestinal and renal excretion of Ca2+ [42].

One of the mechanisms of GA beneficial effects is assumed to be through increasing the fecal nitrogen excretion which in turn reduces serum urea nitrogen levels.

It has been demonstrated that chronic renal failure (CRF) patients consuming low protein diet (LPD) supplemented with 50 g **GA**/d had greater fecal bacterial masses, greater fecal nitrogen excretion, and lower serum urea nitrogen than those consumed the LPD alone or supplemented with 1 g pectin/d. Because elevated concentrations of serum urea nitrogen have been associated with adverse clinical symptoms of CRF, the results suggest that Arabic gum may be a useful adjunct to an LPD for increasing excretion of nitrogenous wastes in feces [43]. Another study by Ali et al. [44] on rat models of acute renal failure showed that **GA** might also improve renal function independently of its action on fecal bacterial ammonia metabolism, but its effect is attributed to a decrease in the generation of free oxygen radicals.

The results of the current research suggested that GA may also improve renal function. The finding of the present research is in harmony with those of [45] who demonstrated that GA had been clinically reported to reduce urea and plasma creatinine concentrations and reduces the need for dialysis from 3 to 2 times per week in chronic renal failure patients [46] GA supplementation significantly increased fecal bacterial mass and fecal nitrogen excretion function.

Our results are in accordance with those of Miranda et al. [47] who illustrated that, LCS treatment in patients with stage 3 and stage 4 chronic renal failure showed a >10% decrease in serum urea concentrations.

The gastrointestinal microflora represents a complex ecosystem containing both beneficial and harmful microorganisms. The composition and function of the intestinal flora is influenced by several factors including age, diet, and antibiotic Treatment.

Chronic kidney failure impacts the intestinal microbiome. Changes in the composition of the intestinal microbiome and disruption of its barrier function may result in the production and absorption of toxic metabolites that can contribute to uremic toxicity, malnutrition, inflammation, and other morbidities in uremic patients [48] (Vaziri et al. 2013). Probiotics modify intestinal microbiome which helps to decrease the bacteria producing uremic toxins [49] (Nakabayashi et al. 2011). chronic kidney disease (CKD) patients have altered gut flora. The alteration of gut flora affects the patients through restoring the gastrointestinal flora balance which favorably impacts the CKD patient and improves any GI issues such as constipation or diarrhea as well as promotes healthy digestion and improved immunity [50].

Chronic renal failure is characterized by progressive retention of several microbial metabolic end products, which are very difficult to be eliminated due to kidney failure and can hardly be removed using dialysis techniques because of their high protein binding [51].

Probiotic was proven to minimize inflammation and oxidative stress in CKD patients. It has been demonstrated that probiotic supplementation in non-dialyzed CKD patients leads to an improvement in the quality of life and a reduction in serum uric acid and creatinine concentrations. Ranganathan et al. [52].

Probiotic is preferred over food sources due to the high potassium, phosphorus, sodium, and sugar content of many foods containing probiotics. In addition, when using quality supplements, the probiotic dose can be accurately estimated and monitored. The preferred dose of probiotic for GI health is to be a blend of bacteria with at least 10 billion colonies forming units took daily [50].

In contrast to the results of the current study, Hyun et al. [53] illustrated that there was no significant effect of probiotics on The reduction of uraemic toxins in pediatric dialysis patients.

There is a decrease in urinary N excretion observed after the administration of GA, LcS or the synbiotic combination of GA and L. casei Shirota.

The combined renoprotective influence of Probiotic *Lactobacillus casei* with Prebiotic **GA** has been demonstrated in the current research where when administered together, they have a profound significant positive influence on renal function.

Increased oxidative stress with impaired antioxidant defense system is considered the major factors leading to the pathogenesis and complications in patients.

It has been recorded that Free radicals are the major cause of various chronic diseases, including diabetes, heart disease, stroke, inflammation and cancer [54]. Tissue injury caused by reactive oxygen species may include DNA damage, protein damage, and oxidation of important enzymes [55] n the human body.

It has been confirmed that the antioxidant harmony comprising SOD, CAT, and GSH considered as the first defense line against the adverse effects of reactive oxygen species .GSH can be converted to GSSG through GPX and converted back to GSH by GR. Thus, changes in the GSH, GSSG, and related enzymatic reactions (GPX and GR) may reflect the antioxidant status [56].

CAT is a hemeprotein enzyme involved in cellular defense, which catalyzes the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals [57].

SOD, a vital enzyme in cellular defense, catalyzes the dismutation of superoxide radicals. [58]. It detoxifies the superoxide anion, thus converting it into H2O2 and water.

In the current study, administration of adenine profoundly decreased ($P<0.05$) the serum and renal activities of these antioxidant enzymes while significantly increasing the serum and renal MDA concentration.

On the other hand, oral administration of GA caused a significant elevation in activities of SOD, CAT, and GSH, associated with significant decline in serum as well as renal levels of malondialdehyde(MDA) production which reflects oxidative stress; which may mirror a decreased antioxidant defense potential.

Thus, our results strongly further suggest the antioxidant activity of GA. The antioxidant properties of GA could be attributed to its constituent flavonoids and other polyphenolics as shown in the Table 2 these phytocomponents have been widely reported to possess antioxidant and anti-lipoperoxidative activities [59].

GA may act either directly by scavenging the reactive oxygen metabolites, because of the presence of various antioxidant compounds or via increasing the synthesis of antioxidant molecules.

Previous research postulated the mechanism of action by which GA improves antioxidant capacity which could be due to the fact that GA contains amino acid residues such as lysine, tyrosine, and histidine, which are generally considered as antioxidants molecules [60].

Ali et al. reported an explanation of the beneficial actions of GA as a dietary supplementation in patients suffering from chronic kidney disease, that GA could ameliorate a loss of antioxidant defense and decrease adenine-induced superoxide production [61].

The results of the present study showed that GA and LcS treatment significantly diminished the oxidative stress induced by adenine administration which indicated by the significant reduction of serum and renal MDA paralleled with a statistically significant increase in serum and renal reduced GSH, SOD and CAT. The best improvement in antioxidant status was observed in group 7 supplemented with both GA and LcS.

Table 4. Serum and urine electrolytes in the tested groups

Parameters / Groups	Serum Na^+ (mmol/ L)	Urinary Na^+ (mmol/L)	Serum K^+ (mmol/ L)	Urinary K^+ (mmol/L)	Serum $Ca2^+$ (mmol/L)
Group 1 Normal control (C)	141.78±3.74^{c}	130.68±5.53^{a}	7.67 ± 0.51 ^{a}	81.18±3.06^{a}	10.19 ± 0.55 ^{a}
Group 2 (A)	165.23 ± 4.15^{a}	179.80±6.27 ^{d}	15.70 ± 0.77 ^{c}	128.24±6.15^{c}	8.56 ± 0.12 ^{c}
Group 3 (GA)	159.51± 4.73^{bd}	132.07±6.56^{a}	7.18 ± 0.51 ^{d}	78.05±6.58^{a}	10.46 ± 0.21 ^{d}
Group 4 (LcS)	163.61± 6.58^{ab}	133.57±6.09^{a}	7.68 ± 0.53 ^{a}	79.17±3.81^{a}	10.21 ± 0.096 ^{a}
Group 5 (A+GA)	152.88 ± 6.98 ^{e}	152.60±6.19b^{c}	10.66 ± 0.60 ^{b}	90.49±4.96^{b}	9.21 ± 0.15 ^{e}
Group 6 (A+ LcS)	155.15 ±5.49 ^{de}	155.97±5.23^{b}	11.18 ± 0.31 ^{e}	88.71±6.41^{b}	10.12 ± 0.178 ^{ab}
Group 7 (A+GA+ LcS)	146.45 ± 5.69 ^{c}	148.80±7.46^{c}	10.44 ± 0.36 ^{b}	83.71±6.38^{c}	9.93 0.24 ^{b}

Values are expressed as means ± SEM *(n = 10). Means with similar superscript (a, b c, d) letters in columns indicate non- significant difference (P< 0.05)*

The major mechanism of the valuable action of GA in adenine-induced CKD might be due to its antioxidative properties. On the other hand, Ali., 2004 demonstrated that gum Arabic did not significantly affect any of the antioxidant enzymes [61].

Probiotics used popularly in humans due to their antimicrobial and antioxidative properties. In addition, Probiotics was considered as cancer prevention agent who might be due to metal particle chelation, compound hindrance, a decrease of ascorbate autoxidation, and ROS searching [62]. The causes of this decline have been suggested to be increased oxidative stress and disorders in energy metabolism, which might participate in important functions [63]. Oxidative stress arises when there is a marked imbalance between the production and the elimination of ROS. results in the urgent formation of free radicals that last for a matter of milliseconds Probiotic have antioxidant activity which improves the function of biochemical reaction in a biological system. Previous research has proposed that specific probiotics assume different biological roles through various mechanisms, [64].

“ROS generation overwhelms antioxidant defenses, and ROS can interact with endogenous macromolecules and change cellular functions” [65]. Elevated level of ROS can also result in protein oxidation (PO) and lipid peroxidation (LPO) which can be used as biomarkers of ROS-induced tissue damage in various diseases [66].

Probiotics may exert their defensive impacts against oxidative stress by reestablishing the gut microbiota]. Probiotic strains can be chosen and explored as promising agents for the prevention and control of several free radical-related disorders [67].

LcS significantly suppress adenine-induced oxidative damage in rats as shown in our results by inhibiting lipid peroxidation and maintaining the antioxidant pool which reflected an increase in the activities of SOD, CAT, and GSH.

In addition to the production of antioxidants and free radical scavenging substances, probiotics also exhibit some important metal chelating activities [68].

Previous in vitro studies demonstrated that Lactobacillus strains to have a vital role in the inactivation of ROS through enzymatic mechanisms based on NADH oxidase/ peroxidase system. Lin and Chang [69].

Lee et al. [70] reported that the antioxidant activity of L. casei is associated with the chelation of metal ions. In addition, lactobacilli are reported to have a vital role in the prevention of the production of OH.

The antioxidative effect of some probiotic strains is emphasized by the inhibition of ascorbate autoxidation, scavenging of free radicals, OH·, and metal ion chelation [71].

Our study in agreements with AOAC [72] which illustrated that lactobacilli have an excessive antioxidant activity while this greatly strained dependent among facultative and obligatory hetero-fermentative lactobacilli. Also, Gao J demonstrated that antioxidant activity of four *Lactobacillus rhamnosus* (B7, B8, B10, B44) was detected by DPPH method. The DPPH scavenging ability of cell-free extracts. The *Lactobacillus rhamnosus* B10 strain showed a relatively high antioxidant activity [73].

Yoghurt bacteria such as *S. salvarius ssp. thermophilus* ATCC 19258 and *L. delbrueckii ssp*. *bulgaricus* ATCC 11842 have been described to have a certain effect on oxidative stress [74]. The peptide hydrolyzed from fermentation could be responsible for minimizing oxidative stress when analyzed for their antioxidant activity by the β-Carotene bleaching assay [75] several studies confirmed antioxidant properties of Lactobacilli [76].

The results of the current study agreed with those of Leila et al. 2015 who showed that Lactobacilli induced a high statistically significant reduction in lipid peroxidation in kidney and liver as well as a reducing in MDA concentration, supporting that Lactobacilli presented effective antioxidative properties and could scavenge the excess of free radicals [77].

A recent study by Kaddam et al. provided more evidence that GA has potent antioxidative effects in humans as demonstrated by its ability to increase TAC and to decrease oxidative stress markers in humans. Thus, the increased intake of dietary antioxidants from GA may help to maintain an adequate antioxidant defense status and consequently contribute to the management of several oxidative stress-related disorders [78]. The Antioxidant Capacity of the combination of

Table 5. Reduced Glutathione, catalase and superoxide dismutase enzymes activity in the tested groups

Parameters / Groups	Erythrocyte SOD activity (U/mL)	Renal SOD (U/100mg)	Blood Reduced glutathione (mg/dL)	Renal Reduced Glutathione (mg/ g tissue)	Serum Catalase (U/ L)	Renal Catalase (U/ g tissue)
Group 1 Normal control (C)	279.46±9.99 a	8.59 ± 0.20 a	15.75 ± 0.61 d	96.43 ± 2.95 a	1593.98 ± 17.06 a	8.29± 0.21 a
Group 2 (A)	185.62±8.79 c	4.60 ± 0.26 d	8.18 ± 0.10 e	60.18 ± 3.29 d	1019.67 ± 20.19 b	3.92 ± 0.31 c
Group 3 (GA)	278.66±9.22 a	8.28 ± 0.26 b	15.23 ± 0.23 a	93.93 ±4.73 a	1599.59± 15.59 a	8.05 ± 0.16 d
Group 4 (LcS)	278.90±11.35 a	8.47 ± 0.28 ab	15.21 ± 0.16 a	94.68 ± 3.78 a	1596.39 ± 19.65 a	8.33 ± 0.12 a
Group 5 (A+GA)	246.98±10.7 b	6.97 ± 0.33 a	11.25 ± 0.41 b	75.37 ± 04.9 bc	1192.97± 12.11 d	5.42 ± 0.24 b
Group 6 (A+ LcS)	249.04±12.06 b	6.37 ± 0.20 e	10.90 ± 0.37 c	72.78 ± 4.43 b	111.39± 14.18 e	5.37 ± 0.18 b
Group 7 (A+GA+ LcS)	257.10±13.23 b	6.78 ± 0.12 c	11.03 ± 0.26 bc	78.68 ± 5.09 c	1218.11 ± 17.63 f	5.52 0.25 b

Values are expressed as means ± SEM *(n = 10). Means with similar superscript (a, b c, d) letters in columns indicate non- significant difference (P< 0.05)*

Table 6. Serum and Renal Malondialdehyde in the tested groups

Parameters / Groups	Serum Malondialdehyde (nmol/ml)	Renal Malondialdehyde (mmol/ g tissue)
Group 1 Normal control (C)	1.82 ± 0.19 ^{ab}	3.46 ± 0.121 ^{a}
Group 2 (A)	4.25 ± 0.086 ^{c}	7.84 ± 0.068 ^{c}
Group 3 (GA)	1.85 ± 0.022 ^{a}	3.41 ± 0.096 ^{ab}
Group 4 (LcS)	1.79 ± 0.038 ^{b}	3.35 ± 0.045 ^{b}
Group 5 (A+GA)	3.09 ± 0.086 ^{d}	5.63 ± 0.153 ^{d}
Group 6 (A+ LcS)	3.20 ± 0.051 ^{d}	5.82± 0.157 ^{d}
Group 7 (A+GA+LcS)	2.58 ± 0.057 ^{e}	5.17 ± 0.127 ^{e}

Values are expressed as means ± SEM (n = 10). Means with similar superscript (a, b c, d) letters in columns indicate non significant difference (P< 0.05)

Probiotic Lactobacillus casei with Prebiotic **GA** has been confirmed in the current study where The administration of gum Arabic (15%, w/v) plus lactobacillus casei shirota 1 x 109 (CFU) resulted in a significant increase in Reduced Glutathione, catalase and superoxide dismutase activities compared to adenine- intoxicated group and also as compared to the individual use of GA or LcS in addition to a significant decrease in peroxidation which might attribute to the prevention of reactive oxygen species generation due to the high phenolic and flavonoid content of GA However, upon fermentation there was increase in the radical scavenging ability. In addition to the reduction of glycation of antioxidant enzymes or reduction of reactive oxygen-free radicals by LcS [79].

4. CONCLUSION

In conclusion, the present study suggests Thus, synbiotic combination of GA and Lactobacillus strains (LcS) has the potential to be a good supplement in order to modify adenine-induced renal toxicity and prevent oxidative stress and associated diseases. Thus, The results suggest that that oral administration of gum Arabic and *Lactobacillus casei Shirota* could conceivably alleviate adverse effects of adenine-induced renal toxicity and can be considered as promising treatment patients with chronic kidney diseases.

ETHICAL APPROVAL

Procedures involving animals and their care were carried out in accordance with international laws and policies (EEC Council directives 86/609, OJL 358, 1 December 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publications No. 85–23, 1985),

BIOETHICAL CLEARANCE

This study was conducted in accordance with academic and ethical approval Experimental procedures conformed to the guidelines provided by the CPCSEA for studies and the ARV resolution on the use of animals in research and to institutional guidelines. The study performed according to the recommendations of, Ain Shams University Research Ethical Committee, (FMASU REC) No. FWA000017585. This study was an animal study and consent to participate and for publication was not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. Kidney Int. 2011;80:1258–1270.
2. Himmelfarb J. Linking oxidative stress and inflammation in kidney disease: Which is the chicken and which is the egg? Semin Dial. 2004;17:449–454.
3. Cheung WW, Paik KH, Mak RH. Inflammation and cachexia in chronic kidney disease. Pediatr Nephrol. 2010;25: 711–312:724.

4. Morena M, Delbosc S, Dupuy AM, Canaud B, Cristol JP. Overproduc- tion of reactive oxygen species in end- stage renal disease patients: A potential component of hemodialysis-associated inflammation. Hemodial Int. 2005;9:37.
5. Ali BH, Al-Husseni I, Beegam S, Al-Shukaili A, Nemmar A, Schierling S, Queisser N, Schupp N. Effect of gumarabic on oxidative stress and in ammation in adenine-induced chronic renal failure in rats. PLoS One. 2013;8:e55242.
6. Goodrum LJ, Patel A, Leykam JF, Kieliszewski MJ. Gum arabic glycoprotein contains glycomodules of bothextensin and arabinogalactan- glycoproteins. Phytochemistry. 2000;54(1):99–106.
7. Dziezak JD. A focus on gums. Food Technol. 1991;45:116–132.
8. Calame W, Weseler AR, Viebke C, Flynn C, Siemensma AD. Gum Arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. Br J Nutr. 2008; 100(6):1269–1275.
9. Huebner ES, Surawicz CM. Probiotics in the prevention and treatment of gastrointestinal infections. Gastroenterol Clin North Am. 2006;35:355-65.
10. Tuohy M, Probert HM, Smejkal CW, Gibson GR. Using probiotics and prebiotics to improve gut health. Drug Discov Today. 2003;8:692–700.
11. Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition adHoc writing committee on the reformulation of the AIN- 76A rodent diet. J. Nutr. 1993;123:1939-1951.
12. AL-Farsi M, Alasalva C, Morris A, Baron M, Shahidi F. Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties growth in Oman. J Agric Food Chem. 2005;53:7592–7599.
13. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999;64:555-559.
14. Yokozawa T, Zheng PD, Oura H, Koizumi F. Animal model of adenine- induced chronic renal failure in rats. Nephron. 1986;44:230–234.
15. Ali BH, Beegam S, Al Lawati I, Waly MI, Nemmar A. Comparative efficacy of three brands of gum arabic on adenine – induced chronic renal failure in rats. Physiological Research: In Press; 2013.
16. Karimi G, Sabran MS, Jamaluddin R, Parvaneh K, Mohtarrudin N, Ahmad Z, Khazaai H, Khodavandi. The anti-obesity effects of Lactobacillus casei strain Shirota versus Orlistat on high fat diet-induced obese rats. Food & Nutrition Research. 2015;59:29273.
17. Guo CH, Huang CJ, Chiou XI, Hsu GSW. Alteration of trace element distribution and testis ACE activity in mice with high peritoneal aluminum, Biol. Trace Elem. Res. 2002;85:145-157.
18. Jaffe M. Concerning the precipitate produced in normal urine by picric acid and a new reaction of creatinine. Z 339 Physiol. Chem. 1986;10:391-400.
19. Bauer JH, Brooks CS, RN. Burch. Clinical appraisal of creatinine clearance as a measurement of glomerular filtration rate. Am. J. Kidney Dis. 1982;2:337–346.
20. Fawecett JK, Scott JE. A rapid and precise method for the determination of urea. J. Clin. Path. 1960;3:156-159.
21. Gochman N, Schmitz JM. Automated determination of uric acid, with use of auricase- peroxidase system. 344 Clin. Chem. 1971;17:1154 –1159.
22. Tietz NW. Fundamentals of clinical chemistry, 2nd ed. Philadelphia; 1997.
23. Batlle D, Chen S, Haque SK. Physiologic principles in the clinical evaluation of electrolyte, water, and acid- base disorders. In: Alpern RJ, Orson WM, Caplan M, eds. Seldin and Giebisch's The Kidney. 5th ed. Philadelphia, PA: Elsevier Saunders. 2013;Chap 74.
24. Chernecky CC, Berger BJ. Sodium, plasma - serum or urine. In: Chernecky CC, Berger BJ, eds. Laboratory Tests and Diagnostic Procedures. 6th ed. Philadelphia, PA: Elsevier Saunders. 2013; 1026-1029.
25. Baginski ES. Method of calcium determination. Clin. Chem. Acta. 1973; 46:49.
26. Ohkawa H, Ohishiand W, Yagi K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. 354 Anal. Biochem. 1979;95:351-358.

27. Minami M, Yoshikana H. A simplified assay method of superoxide dismutase. Clinica Chimica Acta. 1979;92(3):337–356, 342.
28. Beutler E, Duroun O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin 358 Med. 1963; 61:882-8.
29. Superoxide dismutase assays. Methods in Enzymology. 1984;105:93-104.
30. Levesque R. SPSS programming and data management: A guide for SPSS and SAS Users 4th ed. SPSS Inc, Chicago, IL; 2007.
31. Vitetta L, Gobe G. Uremia and chronic kidney disease: The role of the gut microflora and therapies with pro- and prebiotics. Mol. Nutr. Food Res. 2013;57: 824–832.
32. Negri G, Teixeira E, Alves ML, Moreti AC, Otsuk IP, Borguini RG, Salatino A. Hydroxycinnamic acid amide derivatives, phenolic compounds and antioxidant activities of extracts of pollen samples from Southeast Brazil. J Agric Food Chem. 2011;59:5516–5522.
33. A Za'ab M, Al Busadi M, Yas J, Scupp N, Nemmar A, Ali BH. Development of a new model for the induction of chronic kidney disease via intraperitoneal adenine administration, and the effect of treatment with gum acacia thereon. Am J Transl Res. 2015;7(1):28-38.
34. Kandler O, Weiss N. Genus *Lactobacillus*. In: Sneath PHA, Mair NS, Sharpe ME, H JG, eds. Ber ey's Manual of Systematic Bacteriol- ogy, 9th edn. Baltimore: Williams and Wilkins. 1986; 2:1063–5.
    ISBN: 0-683-07893-3.
35. D'Arienzo R, Maurano F, Lavermicocca P, Ricca E, Rossi M. Modulation of the immune response by probiotic strains in a mouse model of gluten sensitivity. Cytokine; 2009;48(3):254-9.
36. Takeuchi K, Tanaka A, Ohno R, Yokota A. Role of COX inhibi- tion in pathogenesis of NSAID-induced small intestinal damage. J Physiol Pharmacol. 2003;54:165–82.
37. Sollid LM, Lie BA. Celiac disease genetics: Current concepts and practical applications. Clin Gastroenterol 376 Hepatol. 2005;3:843–51.
38. Ali BH, Al-Salam S, Al Husseni I, Kayed RR, Al- Masroori N, Al-Harthi T, Al Zaabi M, Nemmar A. Effects of gum Arabic in rats with adenine-in- duced chronic renal failure. Exp Biol Med (May- wood). 2010; 235:373-382.
39. Atsushi K, Noriko H, Masashi T, Hideaki M, Masahirp I. Gum Arabic enhances intestinal calcium absorption in rats. Gum Arabic Enhan.ces Intestinal Calcium Absorption in Rats Journal of Health Science. 2007;53(5):622-624.
40. Ali BH, Ziada A, Blunden G. Biological effects of gum Arabic: A review of some recent research. Food Chem Toxicol. 2009;382:47:1–8.
41. Mahmoud MF, Diaai AA, Ahmed F. Evaluation of the efficacy of ginger, Arabic gum, and Boswellia in acute and chronic renal failure. Ren Fail. 2012;34(1):73–82
42. Nasir O, Umbach AT, Rexhepaj R, Ackermann TF, Bhandaru M, Ebrahim A, Artunc F, Kempe DS, Puchchakayala G, Siraskar B, Foller M, Saeed A, Lanf F. Effects of gum arabic (Acacia Senegal) on renal function in diabetic mice. Kidney 385 Blood Press Res. 2012;35:365-372.
43. Bliss DZ, Stein TP, Schleifer CR, Settle RG. Am. J. Clin. Nutr. 1996;63:392-398.
44. Ali BH, Al-Qarawi AA, Haroun EM, Mousa HM. Renal Failure. 2003;25(1):15-20.
45. Smith CM, Reynard AH. Essentials of pharmacology. Published by Saunders Company. W.B.A. division of Hartcout & Brace Company Philadelphia; 1998.
46. Suliman SM, Hamdouk MI, Elfaki MB. Sudan Association of Physicians, 17th Conference, Friendship Hall, Khartoum, Sudan; 2000.
47. Miranda Alatriste PV, Urbina Arronte R, Gomez Espinosa CO, Espinosa Cuevas Mde L. Effect of probiotics on human blood urea levels in patients with chronic renal failure. Nutricion Hospitalaria. 2014; 29(3):582–590.
48. Vaziri ND, Wong J, Pahl M, Piceno YM, Yuan J, DeSantis TZ, Ni Z, Nguyen TH, & Andersen GL. Chronic kidney disease alters intestinal microbial flora. Kidney International. 2013;83(2):308–315.
49. Nakabayashi I, Nakamura M, Kawakami, K, Ohta T, Kato I, Uchida K, Yoshida M. Effects of synbiotic treatment on serum level of p-cresol in haemodialysis patients: A preliminary study. Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association – European Renal Association. 2011;26(3):1094-1098.
50. Zirker L. The relationship between gut microbiota and CKD: Why use prebiotics

in CKD patients? Ren Nutr Forum. 2014;399(33):1-7.

51. Vanholder R, De Smet R, Glorieux G, Argiles A, Baurmeister U, Brunet P, Clark W, Cohen G, De Deyn PP, Deppisch R, Descamps- Latscha B, Henle T, Jorres A, Lemke HD, Massy ZA, Passlick-Deetjen J, Rodriguez M, Stegmayr B, Stenvinkel P, Tetta C, Wanner C, Zidek W. European uremic toxin work Group. Review on uremic toxins: Classification, concentration, and interindividual variability. Kidney Int. 2003;63:1934–1943.
52. Ranganathan N, Ranganathan P, Friedman EA, et al. Pilot study of probiotic dietary supplementation for promoting healthy kidney function in patients with chronic kidney disease. Adv Ther. 2010;27: 634-647.
53. Hyun HS, Paik KH, Cho HY. p-Cresyl sulfate and indoxyl sulfate in pediatric patients on chronic dialysis. Korean J. 409 Pediatr. 2013;56:159–164.
54. Cheng Z, Li Y, Chang W. Kinetic deoxyribose degradation assay and its application in assessing the antioxidant activities of phenolic compounds in a Fenton-type reaction system. Anal. Chim. Acta. 2003;478:129-137.
55. Gamal El-Din AM, Mostafa AM, Al-Shabanah OA, Al-Bekairi AM, Nagi MN. Protective effect of arabic gum against acetaminophen-induced hepatotoxicity in mice. Pharmacological Research. 2003;48: 631-635.
56. Li Y, Lei K, Xin X, et al. Protective effect of Bacillus subtilis B10 against hydrogen peroxide-induced oxidative stress in a murine macrophage cell line. Int J Agr Biol. 2013;15:927-932.
57. Mamczar EG, Birkner EJZ. Disturbances of kidney function in rats with fluoride-induce hyperglycemia after acute poisoning by sodium fluorid. Fluoride Rese Report. 2005;38(1):48-51.
58. Uboh FE, Akpanabiatu MI, Aquaisua AN, Eno-obong IB. Oral exposure to nitrocellulose thinner solvent induces nephrotoxicity in male albino wistar rats. J Pharmacol Toxicol. 2012;7:78-86.
59. Kassem A. Dietary gum arabic supplementation alter plasma and tissue antioxidant and free radical scavenging activities in Sprague Dawley Male Rats. Journal of Biology and Life Science. 2015;6:129-138.
60. Park EY, Murakami H, Matsumura Y. Effects of the addition of amino acids and peptides on lipid oxidation in a powdery model system. J. Agric. Food Chem. 2005;53:8334-8341.
61. Ali BH. Does gum Arabic have an antioxidant action in rat kidney? Ren Fail. 2004;26(1):1-3.
62. Persichetti E, De Michele A, Codini M, Traina G. Antioxidative capacity of Lactobacillus fermentum LF31 evaluated in vitro by oxygen radical absorbance capacity assay. Nutrition. 2014;30(7-8): 936-8.
63. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature. 2006;443(7113):787-95.
64. Rossi M, Amaretti A. Probiotic properties of bifidobacteria. Norfolk, UK: Caister Academic Press; 2010.
65. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and oxidative stress: A review. Med Sci Monit. 2004; 10(6):Ra141-7.
66. Farmer EE, Mueller MJ. ROS-mediated lipid peroxidation and RES-activated signaling. Annu Rev Plant 424 Biol. 2013;64:429-50.
67. Nardone G, Compare D, Liguori E, Di Mauro V, Rocco A, Barone M, et al. Protective effects of Lactobacillus paracasei F19 in a rat model of oxidative and metabolic hepatic injury. Am J Physiol Gastrointest Liver Physiol. 2010;299(3): G669-76.
68. Spyropoulos BG, Misiakos EP, Fotiadis C, Stoidis CN. Antioxidant properties of probiotics and their protective effects in the pathogenesis of radiation-induced enteritis and colitis. Digestive Diseases and Sciences. 2011;56(2):285-294.
69. Lin MY, Chang FJ. Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and Lactobacillus acidophilus ATCC 4356. Digestive Diseases and Sciences. 2000;45(8):1617-1622.
70. Lee J, Hwang KT, Chung MY, Cho DH, Park CS. Resistance of Lactobacillus casei KCTC 3260 to reactive oxygen species (ROS): Role for a metal ion chelating effect. Journal of Food Science. 2005; 70(8):M388-M391.
71. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M,

Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition. 2012;28(5):539–543.
72. Simpson MR, Dotterud CK, Storro O, Johnsen R, Oien T. Perinatal probiotic supplementation in the prevention ofallergy related disease: 6 year follow up of a randomised controlled trial. BMC Dermatol. 2015;15:13. [DOI] [PubMed]
73. Annuk H, Shchepetova J, Kullisaar T, Songisepp E, Zilmer M, Mikelsaar M. Characte- rization ofintestinal lactobacilli as putative probiotic candidates. J. Appl. Microbiol. 2003;94:403-412. Available:http://dx.doi.org/10.1046/j.1365-432 2672.2003.01847.x
74. Gao J. Antioxidant activity of *Lacbacillus rhamnosus* strains. M.Sc. Thesis, China; 2012.
75. Ou CC, Ko JL, Lin MY. Antioxidative effects of intracellular extracts of yogurt bacteria on peroxidation and intestine 407 cells. Journal of Food and Drug Analysis. 2006;14(3): 304-310.
76. Sakanaka S, Tachibana Y, Ishihara N, Juneja LR. Antioxidant properties of casein calcium peptides and their effects on lipid oxidation in beef homogenates. J Agri. Food Chem. 2005;53:464-468. Available:http://dx.doi.org/10.1021/jf048769 99
77. Mikelsaar M, Zilmer M. *Lactobacillus fermentum* ME-3 – An antimicrobial and antioxidative probiotic. Microb Ecol Heal D. 2009;21:1-27.
78. Bouhafs L, Moudilou EN, Exbrayat JM, Lahouel M, Idoui T. Protective effects of probiotic Lactobacillus plantarum BJ0021 on liver and kidney oxidative stress and apoptosis induced by Endosulfan in pregnant rats. Ren Fail. 2015;37(8):1370-1378.
79. Kaddam L, Fadl-Elmula I, Eisawi OI, Abdelrazig HA, Salih MA, Florian Lang F, Saeed AM. Gum Arabic as novel anti-oxidant agent in sickle cell anemia, phase II trial. Hematology. 2017;17:4. DOI: 10.1186/s12878-017-0075-y 446